M. K. Pinkerton

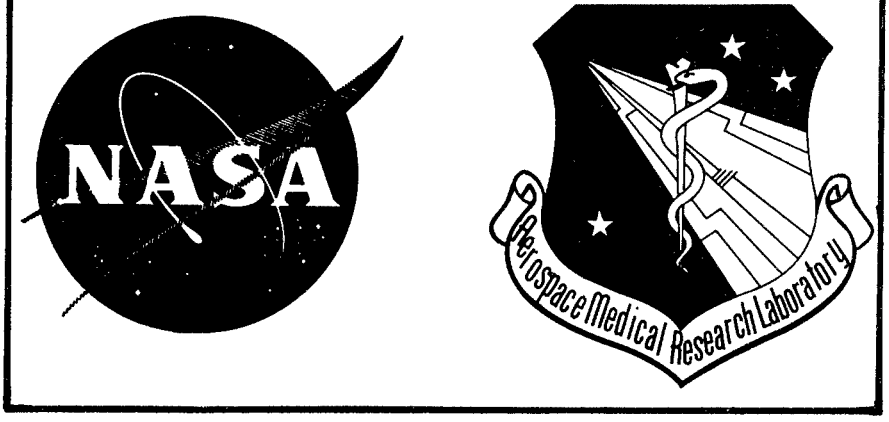
AMRL-TR-72-28
AD 746 395

CONTINUOUS ANIMAL EXPOSURE TO DICHLOROMETHANE

J. D. MacEWEN
E. H. VERNOT
C. C. HAUN
SYSTEMED CORPORATION

MAY 1972

JOINT NASA/USAF STUDY



AEROSPACE MEDICAL RESEARCH LABORATORY
AEROSPACE MEDICAL DIVISION
AIR FORCE SYSTEMS COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO

NOTICES

When US Government drawings, specifications, or other data are used for any purpose other than a definitely related Government procurement operation, the Government thereby incurs no responsibility nor any obligation whatsoever, and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data, is not to be regarded by implication or otherwise, as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

Organizations and individuals receiving announcements or reports via the Aerospace Medical Research Laboratory automatic mailing lists should submit the addressograph plate stamp on the report envelope or refer to the code number when corresponding about change of address or cancellation.

Do not return this copy. Retain or destroy.

Please do not request copies of this report from Aerospace Medical Research Laboratory. Additional copies may be purchased from:

National Technical Information Service
5285 Port Royal Road
Springfield, Virginia 22151

The experiments reported herein were conducted according to the "Guide for Laboratory Animal Facilities and Care," 1965 prepared by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences—National Research Council.

AIR FORCE: 3-8-72/100

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)	2a. REPORT SECURITY CLASSIFICATION
SysteMed Corporation Corporate Headquarters Suite 472 4500 Campus Drive, Newport Beach, Calif. 92660	Unclassified 2b. GROUP N/A

3. REPORT TITLE

CONTINUOUS ANIMAL EXPOSURE TO DICHLOROMETHANE

4. DESCRIPTIVE NOTES *(Type of report and inclusive dates)*

Final Report - January 1972

5. AUTHOR(S) *(First name, middle initial, last name)*

J. D. MacEwen
E. H. Vernot
C. C. Haun

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
May 1972	33	19

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
F33615-70-C-1046 b. PROJECT NO. 6302 c. Task No. 630202 d. Work Unit No. 630202010	SysteMed Corporation Report Number W-71005 9b. OTHER REPORT NO(S) *(Any other numbers that may be assigned this report)* AMRL-TR-72-28

10. DISTRIBUTION STATEMENT

Approved for public release; distribution unlimited

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
Sponsored in part by the National Aeronautics and Space Administration under NASA PR T-80498	Aerospace Medical Research Laboratory, Aerospace Medical Division, Air Force Systems Command, Wright-Patterson AFB, OH 45433

13. ABSTRACT

Continuous exposures of dogs, monkeys, rats and mice to 5000 ppm and 1000 ppm of dichloromethane vapor (CH_2Cl_2) produced severe toxic effects on dogs, rats and mice. Dogs died after 3 weeks exposure to 1000 ppm and after 6 weeks exposure to 5000 ppm. Thirty percent of the mice also succumbed during four weeks exposure to 5000 ppm CH_2Cl_2. Although rats survived 14 weeks exposure to 5000 ppm, they experienced subnormal weight gains. Significant gross and histopathological hepatic lesions were noted in all 3 species at death or experimental termination in 14 weeks. In addition, rats showed abnormal kidney histopathology. Fat stains disclosed mild fatty increase in monkey livers after 14 weeks exposure to 1000 ppm CH_2Cl_2.

Key Words:

Dichloromethane	Dogs
Methylene Chloride	Mice
Toxicology	Monkeys
Space Cabin Contaminants	Rats

DD FORM 1 NOV 65 1473

Security Classification

FOREWORD

This is one of a series of technical reports describing results of the experimental laboratory program being conducted in the toxic Hazards Research Unit. This report is concerned with chronic inhalation toxicity of dichloromethane (CH_2Cl_2), a solvent used in the manufacture of plastic and a common spacecraft contaminant. The research was sponsored by the National Aeronautics and Space Administration under NASA Purchase Request T-80498, funds applied to Air Force Contract F33615-70-C-1046. Work was performed by SysteMed Corporation personnel located at Wright-Patterson Air Force Base, Ohio. K. C. Back, PhD, Chief of the Toxicology Branch, was the technical contract monitor for the Aerospace Medical Research Laboratory.

J. D. MacEwen, PhD, was the principal investigator for the SysteMed Corporation. Acknowledgement is made to W. F. MacKenzie, Lt. Col., USAF, MC, for gross pathological examinations and to R. L. Patrick, MD, of the Laboratory for Experimental Biology, St. Louis, Missouri, for histopathological studies.

This report is designated as SysteMed Corporation Report Number W-71005.

This technical report has been reviewed and is approved.

ANTHONY A. THOMAS, MD
Director
Toxic Hazards Division
Aerospace Medical Research Laboratory

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

SECTION I

INTRODUCTION

Dichloromethane (CH_2Cl_2), also known as methylene chloride, has properties which make it useful as a vehicle for coatings and plastics. It is an excellent solvent and has a vapor pressure of 400 torr at room temperature (75 F). Thus, paint or plastic formulations containing CH_2Cl_2 can be dried faster and under milder conditions than are required for less volatile solvents. Dichloromethane is not considered to be a dangerously toxic material as evidenced by the 8-hour time weighted average limit of 500 ppm quoted in the Federal Occupational Safety and Health Standards of 1971.

Because of these advantageous properties, CH_2Cl_2 has been used extensively in formulating materials used in the Apollo space vehicle and in those materials proposed for the Skylab orbiting space station. Therefore, the possibility exists that relatively large amounts of CH_2Cl_2 might gas off into the closed space cabin atmosphere over long periods of time, eventually leading to high concentrations. NASA requested that continuous inhalation toxicity experiments be run in an effort to define the CH_2Cl_2 levels which might cause toxic signs under these conditions.

A search of the literature revealed that no previous long-term continuous exposures had been performed. However, Heppel and Neal (Reference 5) exposed dogs, rabbits, guinea pigs and rats to 5000 ppm CH_2Cl_2 intermittently, 7 hours a day, 5 days a week for periods up to 6 months. Guinea pigs

were the only species which were adversely affected by the exposure, experiencing subnormal weight gains, decreased food intake and death of 3 of the 8 animals after 35, 90 and 96 exposures, respectively. Examination of the animals that died showed pneumonia and centrilobular fatty degeneration of the liver. In the same study, CNS effects, varying in degree, were produced in the four species previously mentioned, and in monkeys exposed to 10,000 ppm on a 5 day per week, 4 hour per day schedule. Dogs were removed after 6 exposures because of injuries from hyperactivity while all other species finished 36-38 exposures. Lehmann and Schmitt-Kehl (Reference 7) observed only drowsiness and slight reduction in body temperature in cats and rabbits exposed 8 hours a day, 6 days a week to concentrations of 1,728-2,036 ppm for 4 weeks. Little other work has been reported on the chronic toxicity of CH_2Cl_2. Acute LC_{50} values for mice have been reported as 14,500 ppm for a 2-hour exposure (Reference 1) and 16,188 ppm for an 8-hour exposure (Reference 14). Human experience includes the fatality of one of four men accidentally exposed to undetermined concentrations (Reference 9) and the nonfatal exposure of 33 workers to levels of approximately 29-500 ppm (Reference 6).

Consideration of previous work done with CH_2Cl_2 led to the selection of 1000 and 5000 ppm as concentrations which might bracket the toxicity threshold concentration under continuous exposure. An exposure period of 14 weeks was chosen as one which would effectively represent eventual human occupation of the orbiting laboratory and, at the same time reveal any chronic effects of continuous exposure to CH_2Cl_2.

SECTION II

MATERIALS AND METHODS

Animal Exposure Conditions

Exposures were conducted in the Thomas Domes (References 8 and 16) operating in the ambient pressure mode. In these chambers, air flow, pressure, relative humidity and temperature are controlled automatically. Air flow was maintained at 40 cfm, relative humidity at 50 $\pm$ 10% and temperature at 72 $\pm$ 5 F. The absolute pressure was held at 725 torr to seal the chambers and prevent contamination of the surrounding laboratory environment with CH_2Cl_2 vapor.

Technical grade CH_2Cl_2 manufactured by the Dow Chemical Company was used in these studies. Liquid CH_2Cl_2 was delivered from a 10 psi pressurized drum through a flowmeter to a heated glass evaporating flask where it was picked up in a stream of air and carried to the dome. In order to achieve a constant delivery rate of CH_2Cl_2, it was necessary to use a pressure regulator on the air line pressurizing the drum and a flow controller on the line supplying the air which swept out the vaporizer. Continuous analysis of the concentration level in each dome was performed using a flame ionization hydrocarbon analyzer which was calibrated daily with bag samples of known CH_2Cl_2 concentration. The 95% confidence limits of the determination were $\pm$ 50 ppm at both the 5000 and 1000 ppm levels. Measurement over the whole exposure period demonstrated that the contaminant concentrations never varied more than 10% from the nominal values.

Animal Loading

The following groups of animals were placed in each of the exposure domes (1000 and 5000 ppm) and in a control dome where conditions were exactly the same except for contaminant:

400 female ICR mice

20 male Sprague-Dawley rats

8 female beagle dogs

4 female rhesus monkeys

Measurement of Toxic Stress

Mice

One hundred mice from each of the exposure and control domes were to be tested for duration of hexobarbital induced sleep at 30 day intervals from exposure start. Sampling was accomplished by removing 20 mice from each dome every day for a week. The pharmacological basis of this test is the assumption that the duration of sleep is an inverse function of the rate of enzymatic inactivation of the barbiturate. Therefore, decreases or increases in sleep time would be interpreted as activation or inhibition of the responsible enzyme systems by exposure to CH_2Cl_2. The methods used for and results obtained from measurement of sleep time effects have been presented by Van Stee (Reference 18).

High mortality in the 5000 ppm dome caused termination of this exposure, for all species except rats, within 36 days so that only one post-exposure sleep time experiment was carried out. The experiments on the 1000 ppm mice were carried out according to protocol.

On the same sampling schedule - 30, 60 and 90 days - 10 mice were removed from the test and control domes. Immediately after sacrifice, the livers were removed, suspended in sucrose - triethanolamine buffer and frozen at dry ice temperatures. The frozen livers were packed in dry ice and sent air freight to Dr. F. J. Bullock of Arthur D. Little Inc. for determination of cytochromes P-450, P-420 and b_5 (Reference 2).

Twenty mice were housed in a specially constructed activity cage in each of the exposure domes. The cage was placed directly against the dome window so that a television camera connected to a remote activity monitor could view most of the cage. The entire activity measurement system was described by Thomas (Reference 17).

Rats

The 20 rats in each dome were weighed biweekly. After 31 days of exposure, 10 rats exposed to 5000 ppm and 5 control rats were weighed, fasted for 24 hours and sacrificed. Organ weights were measured for calculation of organ/body weight ratios. The rest of the rats in all the domes continued exposure till sacrifice at termination when organ weights were measured as before.

Monkeys and Dogs

Table I lists the clinical test schedule drawn up for the large animals used in this study.

Table I

Clinical Test Schedule for Dogs and Monkeys

	Weeks of Exposure								
	0	2	4	6	8	10	12	13	14
SMA-12 Battery	x							x	
Hematology	x		x		x			x	
Liver Enzymes (SGPT & ICDH)	x		x		x			x	
BSP*	x		x		x			x	
Blood CH_2Cl_2 Level		x							
Body Weight	x	x	x	x	x	x	x		x

*4 monkeys and 4 dogs/dome; 15 min. clearance for dogs; 30 minutes for monkeys

Serum glutamic pyruvic transaminase (SGPT) was measured by the method of Reitman and Frankel (Reference 11) and isocitric dehydrogenase (ICDH) by the Sigma Chemical Company technique (Reference 15). The bromsulphalein test for liver function was that of Rosenthal and White (Reference 12).

Dichloromethane in Blood

Dichloromethane levels in blood were measured by a headspace vaporization procedure developed by the THRU Chemistry Department. It is similar to the method of Divincenzo et al. (Reference 3) which had not yet appeared in the literature when this study began.

Blood was obtained from the test animals and 4 ml immediately injected into each of two partially evacuated 15 ml oxalated test tubes capped with new serum stoppers. One test tube was injected with an additional milliliter of CH_2Cl_2 in water to give a standard addition of 50 ppm. The two test tubes were placed in a 60 ± 1 C water bath for 30 minutes, swirled every 5 minutes, and 2 separate 0.2 ml gas chromatograph injections were made from the headspace of each tube. The syringe was flushed well with air after each injection to remove all CH_2Cl_2, and the needle was cleaned after each injection to prevent clogging by the septum.

Gas chromatographic conditions were as follows:

9" x 1/8" stainless steel Chromosorb 103 column

Column temperature 70 C

Flame ionization detector

Helium carrier gas pressure 15 psig.

The CH_2Cl_2 had a retention time of about 50 seconds, and peak height measurement was used for quantitation.

A water bath temperature of 60 C was chosen to be as high as possible without popping off the stoppers or coagulating the blood. Coagulation causes a rapid decrease in gaseous CH_2Cl_2. At 60 C the amount of CH_2Cl_2 in the gas phase varies only 2% per degree C. Without periodic swirling, recovery is lower and varies more. Calculation of concentration was made by means of simple proportion.

$$x = \text{concentration } CH_2Cl_2 \text{ in blood, ppm}$$

$$\frac{x}{50 + x} = \frac{\text{peak height without addition of standard}}{\text{peak height with addition of standard}} = K$$

$$x = \frac{50\ K}{1-K}$$

The relative standard deviation of the method is $\pm$ 4%.

Formic Acid in Urine

Since Kuzelova and Vlasak (Reference 6) had reported abnormally high concentrations of formic acid in the urine of workers exposed to CH_2Cl_2, it was decided to investigate the occurrence of urinary formic acid of dogs in this study. The method combined a modification of the standard AOAC (Reference 10) steam distillation procedure (75 ml of clarified filtrate was used instead of 150 ml) combined with Grant's (Reference 4) method for the microdetermination of formic acid. Dogs were catheterized after being restrained in specially constructed devices, and the catheters connected to polyethylene bags for the collection of urine. They were then placed in the chamber containing 5000 ppm CH_2Cl_2, and the bags were exchanged for new ones every 6 hours over a 4-day period. Samples were also taken from 2 control dogs exposed to air only.

Gross and histopathologic examinations were made on animals that died or were sacrificed during the study, and on animals that were killed at termination of the study.

SECTION III

EXPERIMENTAL RESULTS

Shortly after initiation of the exposures, it became obvious that they were having an overtly deleterious effect on the test animals. All species became relatively inactive over the first 2 days in the 5000 ppm dome and the dogs, in particular, never regained normal activity. In this experiment from the start, appetite suppression was evident in all animals with dogs again being the most severely affected. Deaths in mice began in the 5000 ppm chamber on the second day of exposure. In the 1000 ppm exposure only the dogs showed signs similar to those seen at the higher concentration. These were pronounced but not as severe as at 5000 ppm CH_2Cl_2.

Mortality

The first large animal to die during exposure was a monkey which expired after 10 days in the 5000 ppm dome. No other monkeys died in the remaining 22 days of this exposure or during exposure to 1000 ppm CH_2Cl_2 for 100 days. Dogs in the 5000 ppm dome died after the following exposure times: one each at days 17 and 21 and 2 more on the 22nd day. After exposure to 5000 ppm for 23 days in the case of dogs and 32 days in the case of monkeys, the remaining animals appeared so weak that it was decided to terminate this portion of the experiment for all test animals except 10 rats

which remained in the chamber for the full 100 days. In the 1000 ppm exposure, dogs died on the following schedule: one each at days 34, 38, 41 and 46 with 2 dying on the 48th day. On the 50th day, the remaining 2 dogs were sacrificed.

Figure 1 is a plot of the number of mouse deaths occurring each day in the 5000 ppm chamber against length of exposure in days. Deaths quickly rose to a maximum of 23 on the third day. Although the succeeding points are scattered, there appears to be an exponential decrease in daily mouse mortality with time from the third day until termination at 36 days when no deaths had occurred in 4 successive days. There is a hint of a possible mortality cycle with a period of 3-5 days, but because of the scatter of the data, no definite conclusions can be drawn in this regard. At any rate, the overall decrease in mortality may indicate that susceptible individuals were being removed from the population or that the remaining animals were developing tolerance to the high concentration of CH_2Cl_2. A total of 10 mice died in the 1000 ppm chamber compared to 3 in the control dome. However, 6 of the exposed mice died on day 40, indicating that they may possibly have been subjected to an unusual trauma at that time. All of the rats survived 100 day exposures to 1000 ppm and 5000 ppm CH_2Cl_2.

Clinical Chemistry

As noted earlier, the dogs and monkeys in the 5000 ppm exposure either died or were sacrificed before the planned blood sampling for clinical chemical tests. In addition, all the dogs in the 1000 ppm exposure were lost or removed within 50 days so that only one sampling for hematology,

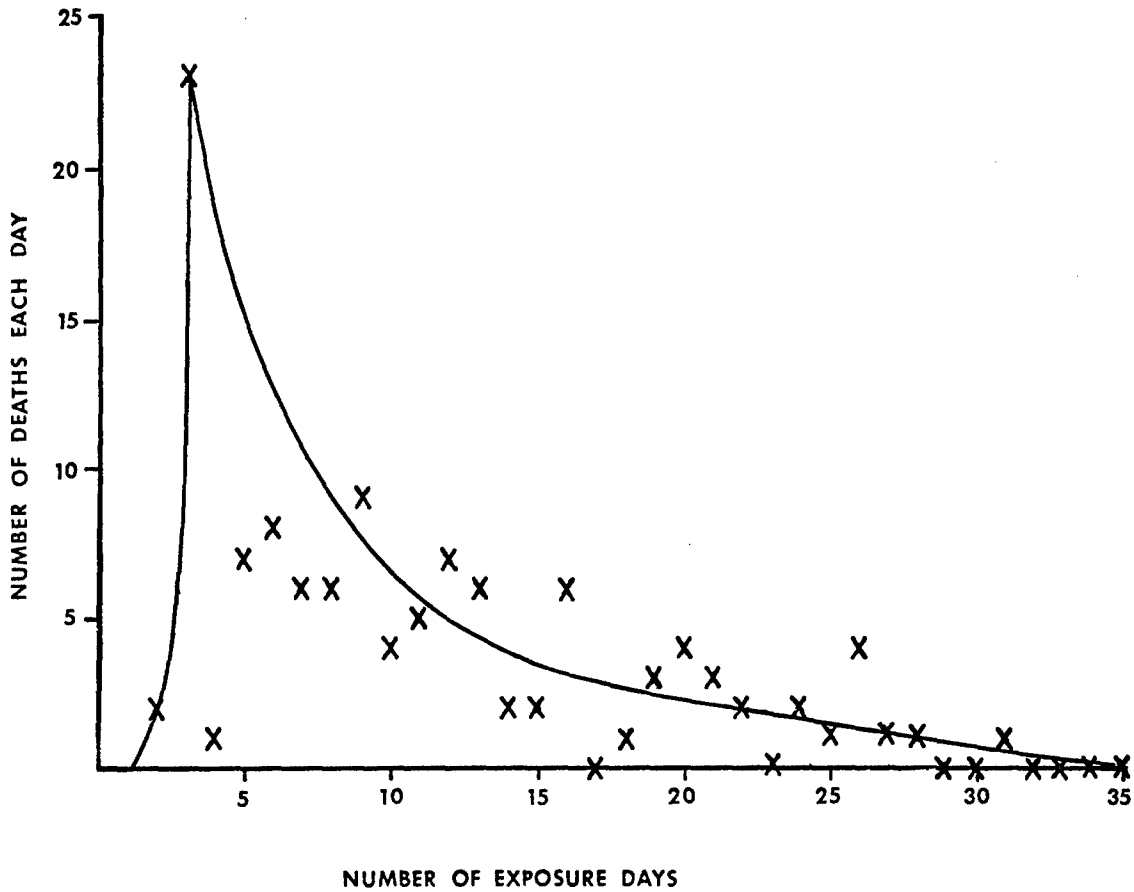


Figure 1

Effect of Length of Exposure to 5000 ppm CH_2Cl_2 on Daily Mouse Mortality

liver enzymes and BSP testing took place. Table II lists the values obtained for the 1000 ppm exposed and control dogs at the preexposure and 4-week sampling periods. The high values for HCT, HGB and RBC may be a reflection of dehydration, since the animals were extremely emaciated at the time of sampling. However, private communications (Reference 13) received since this work was done have indicated that the blood of humans exposed to CH_2Cl_2 contains abnormally high amounts of carboxyhemoglobin. If this is true in dogs, then it is possible that the high hematology values represent the same sort of compensation seen previously (Reference 19) during exposure to high levels of carbon monoxide. The elevated SGPT and ICDH values for exposed dogs indicate that some liver necrosis has taken place in these animals. Although the exposed ICDH difference from control is of borderline significance, the difference from the preexposure value for the same animals is at the 0.01 level, and is more representative of the change due to exposure. BSP values are not changed strikingly by exposure, but this parameter is known to be meaningful only as an indicator of almost complete cessation of liver function.

The monkeys subjected to 1000 ppm CH_2Cl_2 survived the full 100 day exposure so that all scheduled clinical tests were carried out on these animals. The results of hematology, liver enzymes and BSP determinations are shown in Table III. In the case of the monkeys, differences between exposed and controls are nowhere as pronounced as with dogs. The one significant difference between control and exposed ICDH at 4 weeks seems to result from a random fluctuation of the values. There appears to be a trend toward higher

Table II

Effects of 4 Weeks Continuous Exposure to 1000 ppm CH_2Cl_2 on Dog Hematology, Liver Enzymes and BSP[1]

	Preexposure		After 4 Weeks	
	Control	Exposed	Control	Exposed
HCT, vol %	42.8	45.1	41.9	55.5[2]
HGB, g %	14.9	16.0	14.5	18.3[2]
RBC, millions	6.3	6.8	6.3	7.7[2]
WBC, thousands	14.0	11.3	14.0	11.4
Retic., %	0.2	0.6	0.9	0.1[2]
SGPT, Sigma-Frankel Units	33.2	24.9	28.8	102.3[2]
ICDH, Sigma Units	279	188[2]	193	352[3]
BSP, % retention	5.5	6.0	3.25	6.5[3]

(1) Values are means of 8 animals, except for BSP which are means of 4 animals.

(2) Different from control mean at 0.01 significance level.

(3) Different from control mean at 0.05 significance level.

Table III

Effects of Continuous Exposure to 1000 ppm CH_2Cl_2 on Monkey Hematology, Liver Enzymes and BSP[1]

	Preexposure		4 Wks. Exposed		8 Wks. Exposed		13 Wks. Exposed	
	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.
HCT (vol %)	40.8	38.5[2]	40.3	43.3	39.8	43.5[2]	41.0	42.5
HGB (g %)	12.8	12.4	12.9	13.2	13.4	13.0	13.4	13.6
RBC (millions)	5.8	5.6	6.0	6.0	5.8	6.2[3]	5.7	6.0
WBC (thousands)	10.4	12.5	7.9	13.0	8.8	9.7	10.6	7.4
Retic. (%)	0.2	0.7[2]	0.8	1.6	1.3	0.8	1.1	0.8
SGPT (Sigma-Frankel Units)	31.0	29.3	25.5	29.3	31.0	25.8	----	----
ICDH (Sigma Units)	392	379	262	351[2]	344	275	----	----
BSP (% retention)	3.4	5.0	2.8	20.3[2]	8.0	15.0	5.0	9.0

(1) Values are means of 4 animals.
(2) Different from control mean at 0.05 significance level.
(3) Different from control mean at 0.01 significance level.

hematology data in the exposed monkeys but even here, significance is low. Pre- and postexposure monkey blood serum data obtained using the SMA-12 are compared with controls in Table IV. Examination of the table shows that the exposure did not cause these values to change in any biologically significant manner.

Analysis of CH_2Cl_2 in the blood of dogs after 16 days of exposure gave the results shown in Table V. The mean blood concentration in the animals exposed to 5000 ppm CH_2Cl_2, 183 mg/L, was almost exactly 5 times the concentration in the blood of the 1000 ppm exposed animals, 36 mg/L. In order to determine whether detectable amounts of CH_2Cl_2 were excreted unchanged through the kidneys of dogs exposed to 5000 ppm, urine samples from 2 dogs were collected after 6 hours exposure and after 2 days exposure. Analysis revealed 51 mg/L in the first and 33 mg/L in the second sample, demonstrating that there is some excretion of CH_2Cl_2 in this manner.

Urinary formic acid results are presented in Table VI. Since the control and exposed values are essentially identical, it does not appear that dogs metabolize and excrete CH_2Cl_2 as formic acid.

Body and Organ Weights

Large animals experienced severe weight losses during exposures to 5000 ppm CH_2Cl_2. This was also true of dogs in the 1000 ppm chamber. Monkeys exposed to 1000 ppm had static weights over the whole exposure. Dog and monkey body weight data are presented in Table VII.

Table IV

SMA-12 Results on Control and Exposed Monkeys, Continuous 1000 ppm CH_2Cl_2 Exposure for 100 Days[1]

	Preexposure		Postexposure	
	Control	Exposed	Control	Exposed
Sodium, meq/L	154	152	149	150
Potassium, meq/L	4.4	4.6	3.3	3.7[2]
Cholesterol, mg %	153	154	148	135
Calcium, mg %	11.6	11.2	10.0	9.9
Total Phosphorus, mg %	5.3	5.8	5.0	4.3
Total Bilirubin, mg %	0.7	0.6[2]	0.3	0.4
Total Protein, g %	8.0	8.0	6.9	6.8
Uric Acid, mg %	0.6	0.5	0.3	0.4
BUN, mg %	17	18	18	20
Glucose, mg %	98	101	85	72
Alkaline Phosphatase, KA Units	65	72	60	53
SGOT, Sigma-Frankel Units	48	51	54	60
Creatinine, mg %	0.5	0.5	1.2	0.8[2]
Chloride, meq/L	112	111	108	107

(1) Values are means of 4 animals.

(2) Different from control mean at 0.05 significance level.

Table V

CH_2Cl_2 in the Blood of Dogs Exposed Continuously for 16 Days

Individual and Mean Values

5000 ppm Exposure		1000 ppm Exposure	
Dog Number	CH_2Cl_2 in Blood, mg/L	Dog Number	CH_2Cl_2 in Blood, mg/L
M-89	175	N-05	35
N-23	130	N-07	30
N-29	200	N-09	34
N-31	160	N-11	36
N-33	250	N-13	41
Mean	183	N-15	32
		N-19	57
		N-21	25
		Mean	36

Table VI

Urinary Formic Acid in Dogs Exposed to 5000 ppm CH_2Cl_2

Exposure Time Hours	Formic Acid Content, mg/6 hour Sample			
	Control(1)		Exposed	
	N-61	N-65	N-51	N-59(2)
6	0.8	1.2	0.2	0.1
12	1.3	0.9	0.9	0.8
18	1.3	1.2	1.1	0.9
24 (1 day)	2.4	1.5	0.9	0.7
30	0.7	5.1	2.0	---
36	1.3	4.4	1.8	0.6(3)
42	0.6	---	1.3	---
48 (2 days)	1.1	3.0(3)	1.9	2.6(3)
54	0.8	3.4	2.6	---
60			3.2	1.9(3)
66			2.8	
72 (3 days)			1.1	
78			2.0	
84			x(4)	
90			2.1	
96 (4 days)			1.1	
102			1.9	
108			1.8	
6 Hour Mean	1.2	2.6	1.7	1.3

(1) Controls sampled over 54 hours.

(2) Sampling discontinued after 60 hours, cystitis developed due to catherization.

(3) 12-Hour samples

(4) Sample lost

Table VII

Effects of Exposure to CH_2Cl_2 on Dog and Monkey Body Weight

Exposure Length Weeks	Mean Body Weight, Kg[1]					
	Dogs			Monkeys		
	Control	1000 ppm	5000 ppm	Control	1000 ppm	5000 ppm
Pre	8.35	8.28	8.84	2.55	2.36	2.47
2	9.25	7.56	6.02	2.78	2.23	2.18[4]
3			5.67[2] (terminated)			2.00[4] (terminated)
4	9.52	5.86		2.75	2.36	
6	9.56	5.14[3] (terminated)		2.67	2.42	
8	9.80			2.72	2.34	
10	10.39			2.90	2.34	
12	10.55			3.02	2.43	
14	10.59			3.06	2.38	

(1) 8 dogs and 4 monkeys initially in each group.

(2) Mean of 4 dogs.

(3) Mean of 5 dogs.

(4) Mean of 3 monkeys.

The growth rates of the 3 rat groups are shown in Figure 2. Immediately noticeable is the 15 gram mean weight loss by the 5000 ppm exposed rats after 2 weeks. Although 1000 ppm CH_2Cl_2 did not depress rat weight at 2 weeks, from then on the gain rate was significantly lower than for controls and similar to that of the 5000 ppm exposed animals. At termination after 14 weeks, the control rats averaged 35 grams heavier than the 1000 ppm exposed animals and 80 grams heavier than those in the 5000 ppm dome. After 4 weeks exposure, 10 of the 20 rats exposed to 5000 ppm and 5 control animals were sacrificed for determination of organ weights. As shown in Table VIII, none of the exposed organ weights except that of the spleen showed any mathematically significant difference from controls. There is probably no biological significance to the difference in spleen weights since it was no longer evident at 14 weeks. Table IX lists the organ weight data obtained at experimental conclusion. The only organ weights statistically different from controls were those of lungs and kidneys, the kidneys only marginally so. Practically all organ to body weight ratios were significantly higher than controls in rats exposed to 5000 ppm CH_2Cl_2 at both the 4 and 14 week sampling, but this is a reflection of the lower body weight gain in the exposed animals. Since the weight gain rate had not been as severely depressed in the animals exposed to 1000 ppm, the organ to body weight ratios were not different from controls to any biologically significant degree.

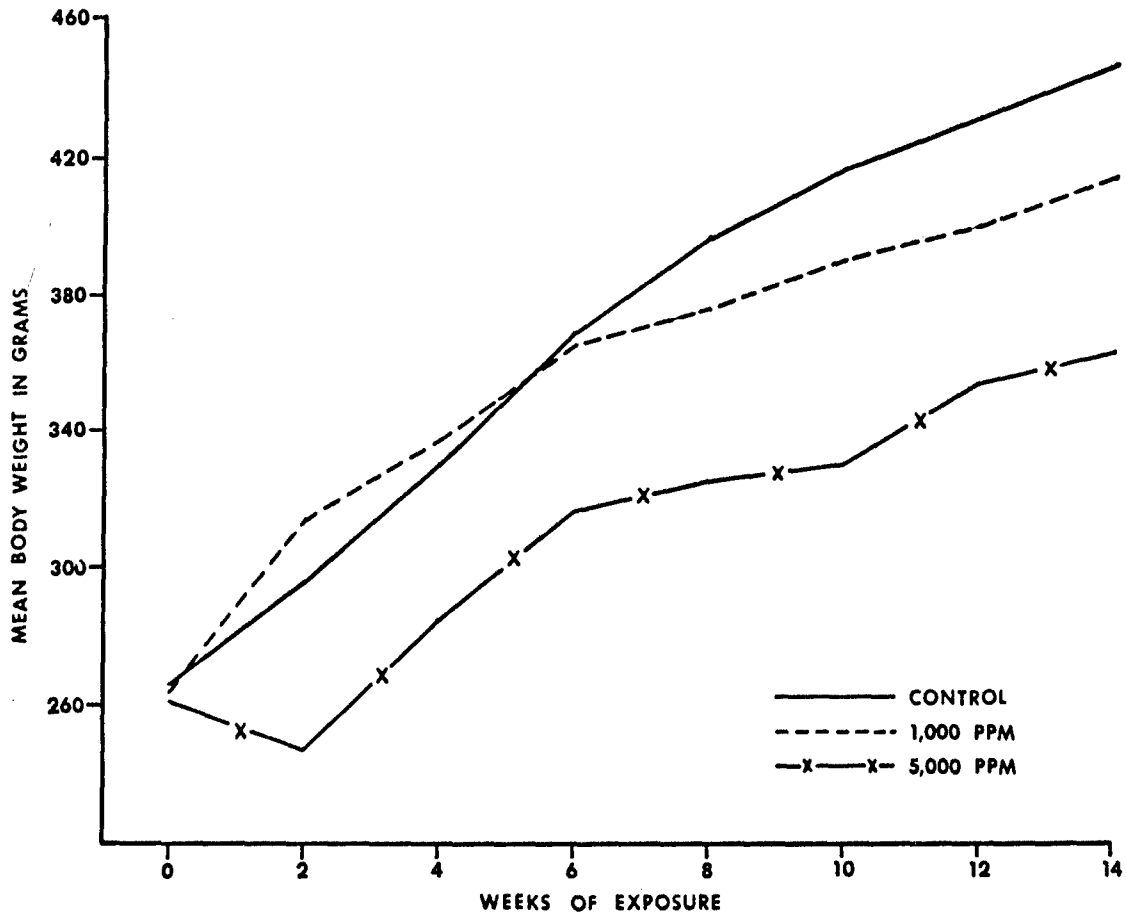


Figure 2

Growth Rates of Rats Exposed to 1000 and 5000 ppm CH_2Cl_2 and Controls

Table VIII

Effect of 4-Week Exposure to 5000 ppm CH_2Cl_2 on Organ Weights of Albino Rats

	Mean Organ Weight (grams)		Mean Organ/Body Weight Ratio (grams/100 grams body weight)	
	Test	Control	Test	Control
	N=10	N=5	N=10	N=5
Heart	1.0	1.0	0.39**	0.34
Lung	1.3	1.3	0.48**	0.42
Liver	8.3	8.0	3.11**	2.65
Spleen	0.5**	0.6	0.20	0.21
Kidney	2.0	2.0	0.74**	0.67

** Different from control mean at the 0.01 significance level.

Table IX

Effect of 14-Week Exposure to 1000 and 5000 ppm on Organ Weights of Albino Rats

	Mean Organ Weight			Mean Organ/Body Weight Ratio		
	1000 ppm	5000 ppm	Control	1000 ppm	5000 ppm	Control
	N=20	N=10	N=15	N=20	N=10	N=15
Heart	1.4	1.3	1.3	0.34*	0.39**	0.31
Lung	1.6	1.5**	1.7	0.40	0.43**	0.40
Liver	10.9	11.2	11.3	2.73	3.23**	2.62
Spleen	0.7	0.7	0.7	0.19	0.20*	0.17
Kidney	2.7	2.4*	2.7	0.67*	0.70**	0.63

* Different from control mean at the 0.05 significance level.

** Different from control at the 0.01 significance level.

Pathology

Gross examination of the dogs that died during exposure or were sacrificed revealed fatty livers and, usually, icterus. Pneumonia was a common finding although not universal. Splenic atrophy was evident in animals dying in the 1000 ppm exposure chamber. An interesting observation was that of edema of the brain or meninges seen in the 4 dogs that died during exposure to 5000 ppm CH_2Cl_2. Monkeys showed no gross lesions on necropsy after 14 weeks exposure to 1000 ppm. Four-week exposure to 5000 ppm produced liver changes described as mild to moderate fatty changes, mild atrophy and swollen liver. Bifrontal encephalomalacia was noted in the one monkey that died. Blotchy livers were found in 4 of 10 rats sacrificed at the end of the 5000 ppm CH_2Cl_2 exposure (100 days). One animal in this group showed white spotted kidneys which were also noted in a rat exposed to 1000 ppm for 14 weeks. None of the rats sacrificed after 4 weeks exposure to 5000 ppm showed any gross abnormalities. This was also true of the mice sacrificed after 4 weeks exposure to 5000 ppm. The livers of mice surviving 14 week exposure to 1000 ppm CH_2Cl_2 showed irregularity of the surface, softening, the impression of loss of parenchyma (atrophy) and a slightly lighter than usual color.

Histopathological examination of H & E and fat stains of dog livers disclosed similar changes in animals exposed to 1000 ppm and 5000 ppm. Marked fatty change involved the entire liver except for a narrow zone of periportal sparing. Most dogs also showed vacuolar change in renal tubules

at the corticomedullary junction. Although the latter effect is occasionally noted in normal dogs, it appeared to occur more frequently and to a more marked degree in these animals. No other histopathological conditions were noted which appeared to be a primary result of the exposure. Although no evidence of cirrhosis was seen, a marked fatty change of the degree seen in dog livers might be expected to lead to cirrhosis in time. No histopathological changes related to CH_2Cl_2 exposure were noted in any of the test monkey tissue which had been H & E stained. However, fat staining tissues from monkeys exposed to 1000 ppm for 14 weeks revealed that 3 of 4 animals had mild fat accumulation abnormally distributed around the central veins.

In addition to H & E staining, special stains were performed on mouse tissues, including Masson trichrome, reticulin, iron and bile stains on liver and iron stains on kidney and spleen. In the mice exposed to 1000 ppm for 14 weeks, hepatic changes were noted in all animals, consisting of the following: rather large masses of brown pigment around portal areas, ductal proliferation in portal areas with focal collapse involving a few cells, occasional pyknotic cells, histiocytic response maximized in the areas of ductal proliferation and around portal areas, nuclear degeneration, and, in some mice, a mild ballooning degeneration of cytoplasm. Nuclear change consisted of variation in size and chromatin clumping.

Reticulin stains showed linear accentuation of reticulin radiating from portal areas of the liver with some increase in collagen. There was a distinct increase in hemosiderin, and the masses of brown pigment cited previously were, at least in part, hemosiderin. All bile stains were negative.

Stains for hemosiderin in kidneys showed very faint granular staining in some tubules of half of the mice examined, None of the control samples stained positive for hemosiderin.

Similar results were obtained in the livers of mice exposed to 5000 ppm CH_2Cl_2 for 4 weeks, the main difference being the absence of ductal proliferation and large pigment masses and lack of disturbance in the reticulum pattern of this group. The amount of stainable hemosiderin is probably lower than in the 1000 ppm exposed animals and distribution is diffuse rather than concentrated in smaller areas. No kidney changes attributable to exposure were noted in these samples.

Rats were the only species to undergo exposure to 5000 ppm CH_2Cl_2 for 14 weeks. There were, therefore, 4 groups of rats examined histologically: 5000 ppm - 4 weeks, 5000 ppm - 14 weeks, 1000 ppm - 14 weeks and controls. Tissues from these animals were subjected to the same stains used on mice. Livers from all 3 exposed groups revealed about the same degree of iron pigmentation in portal areas, cellular vacuolization and nuclear enlargement. However, only one animal exposed to 5000 ppm for 4 weeks exhibited individual cell cytoplasmic degeneration while this phenomenon was present in the great majority of liver tissue from animals exposed to

both concentrations for 14 weeks. There was a general trend toward increase in and condensation of reticulin in all groups. This is believed to indicate cell loss.

The only other rat organ which gave histopathological evidence of exposure effect was the kidney. Iron pigmentation in cortical tubular cells was marginally increased in the 5000 ppm - 4 weeks exposed rat kidneys. This finding was significant in kidneys of animals exposed to both concentrations for 14 weeks. In addition, kidney tissues from the longer term exposure animals demonstrated cortical tubular cell degeneration.

SECTION IV

DISCUSSION

Continuous exposures to 1000 and 5000 ppm dichloromethane were shown to have noxious effects on the 4 animal species used in this experiment. The higher concentration was found to be lethal to 50% of the dogs and 30% of the mice after 3-4 weeks of exposure. Although 1000 ppm did not kill significant numbers of mice over 14 weeks, lethality toward dogs was retained, since 75% of these animals died after 5-7 weeks of exposure. Rats and monkeys experienced depressed weight gains as a result of exposure to CH_2Cl_2.

Elevated SGPT and ICDH values were obtained from dogs after 4 weeks exposure to 1000 ppm. These enzymes are more specific as indicators of liver damage than others such as SGOT and LDH, and the high values obtained point to the liver as being a target organ for the toxic action of CH_2Cl_2 on the dog. Monkey enzymes, contrastingly, showed little if any effect of exposure.

Striking effects of exposure to CH_2Cl_2 were revealed by histopathologic examination. The dogs in both exposures demonstrated marked fatty change which might have been expected to result in cirrhosis if longer exposure times had been possible. It must be recalled that this and other dog liver changes had occurred after only 3 weeks of exposure to 5000 ppm and 6-7 weeks exposure to 1000 ppm. Mice sacrificed after 5 weeks exposure to 5000 ppm CH_2Cl_2 had liver changes in nuclei, cytoplasm and cell organization. After 14 weeks exposure to 1000 ppm, these observations were again present and had progressed

to the point where focal collapse and ductal proliferation indicated an early stage of cirrhosis.

Significant histopathological liver changes were noted in all rat groups exposed to CH_2Cl_2, and there was obvious cellular cytoplasmic degeneration in animals exposed to both concentrations for 14 weeks. There was histological evidence of renal toxicity in the rat with the presence of iron pigmentation in cortical tubular cells. After 14 weeks exposure, cortical tubular cell degeneration was apparent. The pronounced gross and histopathological changes noted in rat livers and kidneys were not accompanied by any significant differences in organ weights from control values. This is an unusual finding, since overt evidence of gross change in an organ is usually accompanied or preceded by organ weight change.

Except for the one animal that died early in the 5000 ppm exposure, little pathological indication of toxicity toward monkeys was obtained. Gross observations of mild to moderate liver changes were made in the animals exposed to 5000 ppm CH_2Cl_2, but no lesions were noted on examination of the animals after 14 weeks exposure to 1000 ppm. H & E stains of exposed monkey tissue was negative although some liver fat accumulation was noted after fat staining tissues of 14 week - 1000 ppm exposed animals.

Overall, the evidence developed indicates that, under the conditions of this experiment, CH_2Cl_2 is toxic to all species tested, most to dogs, less so to mice and rats and least to rhesus monkeys.

REFERENCES

1. Browning, E., Toxicity and Metabolism of Industrial Solvents, Elsevier Publishing Company, 243, 1965.

2. Bullock, F. J., M. Callahan and E. S. Harris, "A Study of Liver Microsomal Cytochromes Following Chronic Exposure to Dichloro-Methane," Proceedings of the Second Conference on Environmental Toxicology, AMRL-71-120, Aerospace Medical Research Laboratory, Wright-Patterson AFB, Ohio, In Press.

3. Divincenzo, G. D., F. J. Yamo and B. D. Cestill, "The Gas Chromatographic Analysis of Methylene Chloride in Breath, Blood and Urine," Am. Ind. Hyg. Assoc. J., 32:387, 1971.

4. Grant, W. M., "Colorimetric Microdetermination of Formic Acid Based on Reduction to Formaldehyde," Anal. Chem., 20:267, 1948.

5. Heppel, L. A., P. A. Neal, T. L. Perrin, M. L. Orr and V. T. Porterfield, "Toxicology of Dichloromethane (Methylene Chloride) I. Studies on Effects of Daily Inhalation," Ind. Hyg. Toxicol., 26:8, 1944.

6. Kuzelova, M. and R. Vlasak, "The Effect of Dichloromethane on the Health of Workers Engaged in the Manufacture of Plastic Film and the Determination of Formic Acid as a Metabolite of Dichloromethane," Pracovni lekarstvi, Prague, Czechloslovakia, 18:167, 1966. Abstracted in Occup. Safety and Health Abst., 5:167, 1966.

7. Lehmann, K. B and L. Schmidt-Kehl, "The Thirteen Most Important Chlorinated Aliphatic Hydrocarbons from the Standpoint of Industrial Hygiene," Arch. F. Hyg., 16:131, 1936.

8. MacEwen, J. D., Toxic Hazards Research Unit Design and Construction Phase, AMRL-TR-65-125, Aerospace Medical Research Laboratory, Wright-Patterson AFB, Ohio, September, 1965.

9. Moskovitz, S and H. Shapiro, "Fatal Exposure to Methylene Chloride Vapor," A.M.A. Arch. Ind. Hyg. Occup. Med., 6:116, 1952.

10. Official Methods of Analysis, W. Horwitz, Editor, Page 274, Assoc. of Official Agricultural Chemists, 1965.

11. Reitman, S. and S. Frankel, "A Colorimetric Method for the Determination of Serum Glutamic-Oxalacetic and Glutamic - Pyruvic Transaminases," Am. J. Clin. Path., 28:56-63, 1957.

12. Rosenthal, S. M. and E. L. White, "Clinical Application of the Bromsulphalein Test for Hepatic Function," J. A. M. A., 84:1112, 1925.

13. Stewart, R. D., Personal Communication.

14. Svirbely, J. L., B. Highman, W. C. Alford and W. F. Von Oettingen, "The Toxicity and Narcotic Action of Monochloro-monobromomethane with Special Reference to Inorganic and Volatile Bromide in Blood, Urine and Brain," J. Ind. Hyg. Toxicol., 29:382, 1947.

15. Technical Bulletin Number 175, "The Colorimetric Determination of Isocitric Dehydrogenase in Serum or Other Fluids," Sigma Chemical Company, December, 1967.

16. Thomas, A. A., "Low Ambient Pressure Environments and Toxicity," A.M.A. Arch. Environ. Health, 11:316, 1968.

17. Thomas, A. A., "Effects of Methylene Chloride Exposure on the Spontaneous Activity of Mice," Proceedings of the Second Conference on Environmental Toxicology, AMRL-71-120, Aerospace Medical Research Laboratory, Wright-Patterson AFB, Ohio, In Press.

18. Van Stee, E. W., J. P. F. Murphy and K. C. Back, "Halogenated Hydrocarbons and Drug Metabolism: The Effect of Fluorocarbons on Hexobarbital Sleeping and Zoxazolamine Paralysis Times in Mice," Proceedings of the Second Conference on Environmental Toxicology, AMRL-71-120, Aerospace Medical Research Laboratory, Wright-Patterson AFB, Ohio, In Press.

19. Vernot, E. H., W. F. MacKenzie, J. D. MacEwen, P. N. Monteleone, M. E. George, P. M. Chikos, K. C. Back, A. A. Thomas and C. C. Haun, "Hematological Effects of Long-Term Continuous Animal Exposure to Carbon Monoxide," Proceedings of the First Conference on Environmental Toxicology, AMRL-TR-70-102, Wright-Patterson AFB, Ohio, December, 1970.

☆U.S.Government Printing Office: 1972 — 759-087/737